EJFA Emirates Journal of Food and Agriculture

Emirates Journal of Food and Agriculture 36: 1–13
doi: 10.3897/ejfa.2024.127665

RESEARCH PAPER

Insights into physiological and biochemical responses of *Zea mays* L. under salinity stress

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Academic editor: Fernando Lidon ♦ **Received** 16 May 2024 ♦ **Accepted** 14 August 2024 ♦ **Published** 9 September 2024

Abstract

Growth of crops in the semi-arid and arid regions of the world is significantly affected by salinity stress. The present study investigated maize, which is a globally cultivated crop and known for its salt sensitivity. Widespread cultivation and notable characteristics of maize make it an exemplary model for investigating stress response. We analyzed maize plants morphological and biochemical responses under salinity stress at different intervals. Current results showed that under 100 mM NaCl stress, germination rate of maize seeds decreases up to 40% compared to their respective control non-stressed seeds. Additionally, prolonged exposure to salinity stress negatively impacted various physiological and biochemical aspects of maize plants including shoot/root length, leaf width, and chlorophyll contents, especially in plants treated with NaCl for 21 days. Furthermore, our findings showed that the NaCl stress triggered various enzymatic and non-enzymatic activities, including catalase (CAT), polyphenol oxidase (PPO), total polyphenol (TPP), and flavonoid in maize plants. However, maize plants respond differently to NaCl stress regarding protein content in different durations. This research underscores the urgent need for innovative strategies to mitigate salinity stress and food security for local maize varieties in saline regions and provides valuable insights to tackle these challenges.

Keywords

Abiotic stresses, Salinity, Sustainable agriculture, *Zea mays*, CAT, PPO, TPP, Flavonoids contents

Introduction

Salinity has been identified as a significant abiotic stress that harms agricultural yields, particularly in regions having arid and semi-arid climates (Ashrafi et al. 2018). As the world population grows, food production must increase by 70% by 2050, placing immense pressure on food producers (Shabala 2013). Salinity impacts plant morphology and physiology, impeding plant development and ultimately diminishing agricultural productivity (Abbasi et al. 2012). Almost one billion hectares of agricultural land is influenced by salinity, and one-fifth of the irrigated areas are impacted by salinity which leads to significant economic losses (Zaman et al. 2018). In the presence of elevated soil salinity, the osmotic potential of plant cells becomes significantly negative. Thus, the osmotic

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gradient facilitates water movement out of the plant cells, consequently decreasing turgor pressure (Betzen et al. 2019). The combined impact of physiological drought and ion toxicity, salinity negatively impacts root functionality, consequently affecting the plant's ability to absorb water (Eraslan et al. 2007; Shah et al. 2023). Plant tissues with elevated Na^+ levels induce oxidative damage of the membranes by producing reactive oxygen species (ROS) during various biochemical processes (Chinnusamy et al. 2005). Significantly a 0.25 M concentration of NaCl can have detrimental effects on maize plants including decreasing growth and wilting (de Azevedo Neto et al. 2006). Plants growing in saline soil experience abnormalities at phenotypic as well at physiological levels, which causes ionic imbalances, osmotic and ionic stress, inhibits important metabolic processes, and results in decreased productivity (Munns and Tester 2008). As tissue osmotic balance and water potential decline, vital plant functions, including growth, photosynthesis, and protein synthesis are compromised. Additionally, salt stress reduces root and shoot development (Meloni et al. 2001). It also reduces chlorophyll content and leaf area, thus reducing photosynthesis (Tezara et al. 1999). Numerous crops exhibit various physiological, morphological, and biochemical responses to salinity stress (Aras et al. 2019). A major global food crop, maize (*Zea mays* L.) is used as the primary raw material in a variety of industries (Meng et al. 2016). Numerous research studies have demonstrated that maize productivity should be increased by 100% to address the growing demand for human and animal consumptions and biofuel production, especially in underdeveloped and developing nations (Nuss and Tanumihardjo 2010). Maize is recognized as a plant with moderate sensitivity to salt, and its production are significantly constrained under NaCl stress (Farooq et al. 2015). While excess amount of salt significantly hinders plants growth and production. Moreover, it has been observed that many areas where major corn production occurs are impacted by high soil salinization due to environmental change, water shortages, and other reasons. Salinity stress has been recognized as one of the significant threats to maize yield in recent years (Farooq et al. 2015). Current studies to examine physiological factors under constant treatment conditions over extended periods. Our study examines how physiological and biochemical parameters vary throughout a maize cultivar's developmental stages. A lack of knowledge regarding the biochemical and physiological responses to salt stress of local cultivar with different exposure durations highlights the importance of the research. As a result of our findings, we will likely uncover mechanisms that enhance maize resilience to saline stress, leading to the advancement of breeding programs for salt-tolerant grains. Our aim is to address significant knowledge gaps concerning the interaction between maize physiology and biochemistry during salt stress throughout various developmental stages. Our study sheds light on the mechanisms that make maize resilient to salinity stress, paving the way for developing more resilient varieties. To contribute to global food security, breeding programs need to maximize the benefits of these insights to fortify maize and other essential grain crops against soil salinity. We cannot understate how important it is to enhance crop resilience to abiotic stresses like salinity within the next few decades to feed an ever-growing global population. Through collaborative efforts and innovative research, agriculture can have the knowledge and tools it needs for sustainable and sufficient food production.

Methodology

Experimental layout

Maize seeds were collected from the KPK Agricultural Research Centre in Peshawar and 0.1% $HgCl_2$, and 70% Ethanol was used for seeds sterilization. After five days, the seeds were transferred to petri plates and 4 mL of diluted water was added then after 4 days. The germinated seedlings were placed in 10-by-9-cm plastic containers mixed with agricultural substrate which included of 10–15% coco peat moss, 45–50% coco peat, and 35–40% perlite, along with essential nutrients such as; approximately 0.09 mg/g of NH_4^+, about 0.1 mg/g of K_2O, 6–8% zeolite, about 0.205 mg/g, of NO^{-3}, and about 0.35 mg/g, of PO_4^{+3}. This experiment used different periods with 100 mM stress conditions on maize plants. (a) Control group, with only dH_2O added (b) 7 days control. (c) 7 days with 100 mM NaCl (d) 14 days control. (e) 14 days with 100 mM NaCl (g) 21 days control and (h) 21 days with 100 mM NaCl. The prepared pots were kept to growth chamber maintained at a temperature of 28 °C, with an 8/16 hour period of light and dark, and 55–65% relative humidity.

Assessment of seed germination assay

The germination percentage of both the control and the 100 mM NaCl treatments after 7 days was measured by given method (Carpýcý et al. 2009).

$$\text{GP (\%) Germination percentage} = \frac{\text{no of germinated seeds}}{\textit{no of toal seeds}} \times 100$$

Plant biomass and leaf measurements

The maize plants were harvested after 30 days and both roots and shoots were recorded. For further examination, the collected samples were stored at -80 °C. Initially, the fresh weight (FW) of the uppermost maize leaves was measured. Secondly, the leaves were kept in sealed tubes including DI water for 6 hours to measure their turgid weight (TW). Finally, the leaves were dried for 48 hours at 80 °C in an oven, and recorded their dry weights (Carpýcý et al. 2009). RWC and leaf biomasses (Elings 2000) were measured by formula.

$$\text{FW-DW/TW-DW} \times 100 = \text{RWC (\%)}$$

$$\text{Leaf area} = L \times W \times A$$

Chlorophyll contents measurement

Fresh maize plant leaves were crushed using liquid nitrogen, and 200 mg of the resulting ground powder was added to a 1.5 ml tube. Next, 1 ml of 80% acetone was added, followed by brief vortexing to ensure thorough mixing. The tube was centrifuged at 1000 rpm for five minutes and then50 to 100 µL centrifuged sample was transferred to the well, and the spectrometer measured chlorophyll a, chlorophyll b, and carotenoid at 663 nm, 645 nm, and 470 nm wavelengths. The concentrations of all photosynthetic pigments were determined using the extension coefficient and an equation derived from the Barnes method with minor adjustments (Barnes et al. 1992).

Protein contents measurement

The Bradford technique was used to determine the protein content with some slight adjustments (Bradford 1976). According to Arulsekar and Parfitt's formulation, 200 mg of ground leaf samples from each group was put in 1 mL of an extraction buffer (Arulsekar and Parfitt 1986). Samples were thoroughly homogenized, centrifuged at 4000 rpm for 10 minutes at 2 °C, and 150 µL Bradford reagent was added to fresh 1.5 mL tubes. After five minutes of incubation at room temperature, 150 µl of the final mixture from each treatment group was pipetted into 96-well microplates. The absorbance at 595 nm was determined with an xMark™ microplate reader.

Catalase contents measurements

A frozen leaf was ground into a fine powder using liquid nitrogen to determine enzyme activity. An extraction buffer of 50 mM Tris at pH 7.0, 10% glycerol, 3 mM magnesium chloride, 1 mM EDTA, and 1% PVP was added to the powder. The mixture was centrifuged for 15 minutes at 10,000 rpm at 4 °C and then 240 µL of the obtained supernatant was immediately mixed with 120 µL of 0.2 M H_2O_2 and 0.1 mM phosphate buffer (pH 7.0). The optical density at a specific wavelength of 240 nm was measured using a spectrophotometer.

Total flavonoid content measurement

Total contents measured by the Park method (Park et al. 2008). Fresh leaf samples were meticulously ground using a mortar and pestle with the aid of liquid nitrogen. Subsequently, 0.5 g of the resulting powder was blended with 1 mL of 80% methanol, and this mixture was left at room temperature for duration of 24 hours. Following this incubation period, the mixture centrifugated for 15 minutes at a speed of 10,000 rpm. The resulting supernatant was then gathered and combined with an equal volume of 2% $AlCl_3$ solution, which had been diluted in 95% ethanol. After a 20 minute incubation at room temperature, the absorbance was measured spectrophotometrically at a wavelength of 390 nm.

Total phenol oxidase contents measurement

A phenolic content of maize was examined by Folin-Ciocalteu method (Sánchez-Rangel et al. 2013). Crushed 5 g samples in a tube, mixed it with 150 µL extract from each sample, quickly added with 1.5 mL of 2% Na_2CO_3, and gently vortexed for 3 minutes. Them 0.1 mL of $CuSO_4$ and sodium and potassium tartrate were mixed, mixture was kept in the dark for 5 min at room temperature then centrifuged at room temperature for 10 minutes at 10,000 rpm and measured at 750 nm (Ullah et al. 2019b).

Polyphenol oxidase contents measurement

In a previous study (Kumar and PA 1982), 2.9 mL of substrate solution (0.05 M catechol solution produced in a 0.1 M phosphate buffer with pH 6.5) was mixed with 100 µL of sample from each treatment group. The resulting mixture was kept in a water bath at 30 °C. Using spectrophotometry, the oxidation of catechol was measured at 420 nm. As a blank, the catechol solution was utilized.

Statistical analysis

Five replicates were used for experiments that generated relevant data, whose pool included analysis. Following the performance of one-way ANOVA along with Duncan's multiple range test (DMRT), treatments can be evaluated based on their variations. Treatment averages were assessed using an unsystematic design.

Results

Seeds germination rate

It is well documented that excessive Na^+ and Cl^- ion accumulations adversely affect seed growth (Hasanuzzaman and Fujita 2013). Compared to the control group, our statistical analysis showed that NaCl significantly reduced the germination rate of maize seeds (Fig. 1A), the untreated plates demonstrated the highest seed germination rate, while NaCl-treated plates showed a significant decline. Fig. 1B illustrates how 100 mM NaCl salt stress reduced the germination of seeds by 40%. The adverse effect of salt stress on the growth of maize seedlings is illustrated by a

Figure 1. Compared to the control and salt stress maize germination rate (**A**). Growth percentage under 100 mM NaCl stress (**B**). Based on the DMRT, bars with distinct tiny letters denote significant differences ($p < 0.05$) between treated and control plants that experienced different lengths of salt stress.

decreased germination rate. Because NaCl induced high salinity levels, the seeds were subject to osmotic stress, which inhibited water uptake and consequently impacted germination. Furthermore, the specific ion effect of Na^+ and Cl^- ions disrupted the delicate balance of nutrients essential to seedling growth. A strategy for enhancing seedling resilience to salt stress is critical, especially in regions where soil salinity poses significant agricultural challenges.

Plant root and shoot analysis

Various plant growth parameters were consistently and progressively negatively impacted by 100 mM salt concentration in different timeframes (Fig. 2A–C). The growth metrics of maize plants declined continuously over the exposure periods of 7 to 14 and 21 days. It was found that 7 days after salt stress treatment, roots displayed a significant reduction of 29% compared to control plants. The reduction intensified to 57.1 and 68.7% after 14 and 21 days of salt stress treatment. we noted 38 and 60% of root length increased by 14 and 21 days compared to control plants (Fig. 2A). Moreover, after 7, 14, and 21 days of salt treatment, salt-treated plants significantly reduced shoot length compared to controls by 17, 33, and 46%, respectively (Fig. 2B). Untreated 21 days control plants showed a remarkable 29.3% induce in shoot length compared to the untreated 7- and 14-days controls, indicating prolonged salt exposure adversely affects the growth of both roots and shoots.

RWC and leaf biomass

Due to 100 mM salt concentration, leaf biomass and relative water content (RWC) showed dynamic trends over 7, 14, and 21 days (Fig. 3A, B). After 21 days of treatment, leaf biomass was markedly reduced by 47.4% compared to control plants, while 7 and 14 day treated plants showed 12 and 27.6% reductions in RWC, respectively. Compared to the 7 and 14 days controls, the 21 days plant exhibited a 35.1% increase in RWC; the treated plants experienced an 18.9% decrease (Fig. 3B). The length and width of maize leaves were significantly reduced in treated plants, but their dimensions increased significantly in untreated maize plants (Fig. 3C, D). A drastic reduction in leaf length and width was observed after 21 days of treatment compared to a control group, with significant decreases of 53% and 71%, respectively. Compared to 7 and 14 days controls, 21 days control plant increased leaf length and width by 40 and 35.4%, respectively. The leaf dimensions of 7 and 14 treated plants decreased by 12.1, 34.4, 22.5%, and 50%, respectively. Interestingly, control plants' leaf areas initially reduced and then increased after 7, 14, and 21 days, whereas treated plants consistently decreased over the same period under 100 mM NaCl. On the 21 days treated plants exhibited a significant reduction in leaf area by 86.8% and 63% compared to plants treated for 7 or 14 days. On the other hand, the 21 days control plants exhibited a remarkable 90.4% increase in leaf area compared to the 7 and 14 days control plants (Fig. 3E).

Chlorophyll contents

A study of chlorophyll concentrations in maize plants (Fig. 4A–C) revealed consistent decreases in chlorophyll a, b, and carotenoid content with prolonged exposure to NaCl (7, 14, and 21 days). Comparatively, in the control plant, chlorophyll a, b, and carotenoids reduced by 12.8, 7.1, and 7.7% after 7 days of salt stress, respectively. Reduction of chlorophyll a, b, and carotenoids contents occurred at 14 days, ranging from 7.1, 20.4, and 22.6%. Compared to the control, chlorophyll a, b, and carotenoids decreased most significantly after 21 days of salt treatment, by 29.8, 22.6, and 62%, respectively. A 21 day treatment resulted in a significant reduction of 16.1, 25.6, and 62% in chlorophyll a, b, and carotenoid levels, respectively, compared to a 7 or 14 day treatment. The control plants showed 61.7% higher carotenoid content at 21 days compared with different chlorophyll b and control periods. It is clear from these findings how salt stress affects chlorophyll pigments over time, and the complex relationship between stress duration and pigment content has implications for plant photosynthetic capacity and resilience to stress.

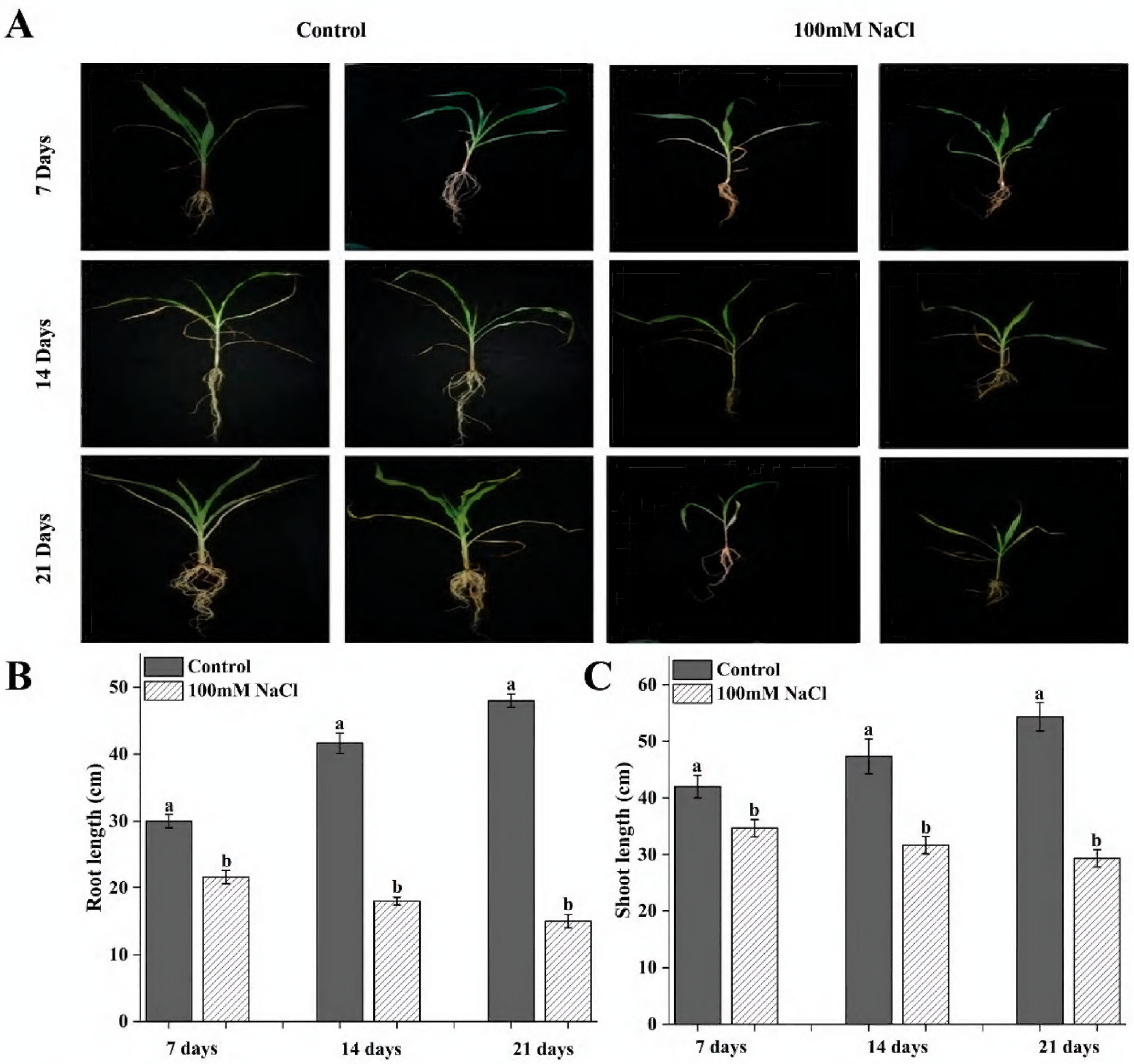

Figure 2. Maize plants morphologically after 7, 14, and 21 days under 100 mM NaCl (**A**). Root length (**B**). Shoot length (**C**). Based on the DMRT, bars with distinct tiny letters denote significant differences ($p < 0.05$) between treated and control plants that experienced different lengths of salt stress.

Catalase and protein contents

A detailed analysis of catalase and protein contents (Fig. 5A, B) over different timeframes (7, 14, and 21 days) revealed intriguing dynamics. A significant induced in protein contents were reported in the control, while catalase content increased for extended days. In 14 and 21-day control plants, the catalase contents were induced by 1.5 and 5.4% compared to 7 days. As a result, 21 days salt-treated plants significantly had 9.1% higher catalase contents than plants that had been salt-stressed for 7 and 14 days. However, with varying exposure durations to the same salt concentration, catalase contents consistently increased by 2.9, 5.5, and 6.5% compared to the control. Notably, protein content was significantly increased with 100 mM salt stress at different periods. Protein contents observed 5.3 and 11.1% reduced compared to 14 and 21 days of control plants. These findings illuminate the intricate response mechanisms of protein and catalase contents to salt stress over time, suggesting potential adaptive strategies employed by the plant to mitigate the stress impact and highlighting the nuanced interaction between stress duration and these biochemical parameters.

Polyphenol oxidase, total polyphenol, and flavonoid contents

Fig. 6 showed the contents of polyphenol oxidase (PPO), total polyphenol, and flavonoid of maize plants grown under 100 mM salt concentration over different periods (7, 14 days had substantial increases in TPP content, respectively, of 38 and 28.6%. Compared to the control, 14 day plants showed the highest total flavanol content 66.2% (Fig. 6C), Our results showed flavonoid content significantly 90 and 96% increased by 14 and 21 days

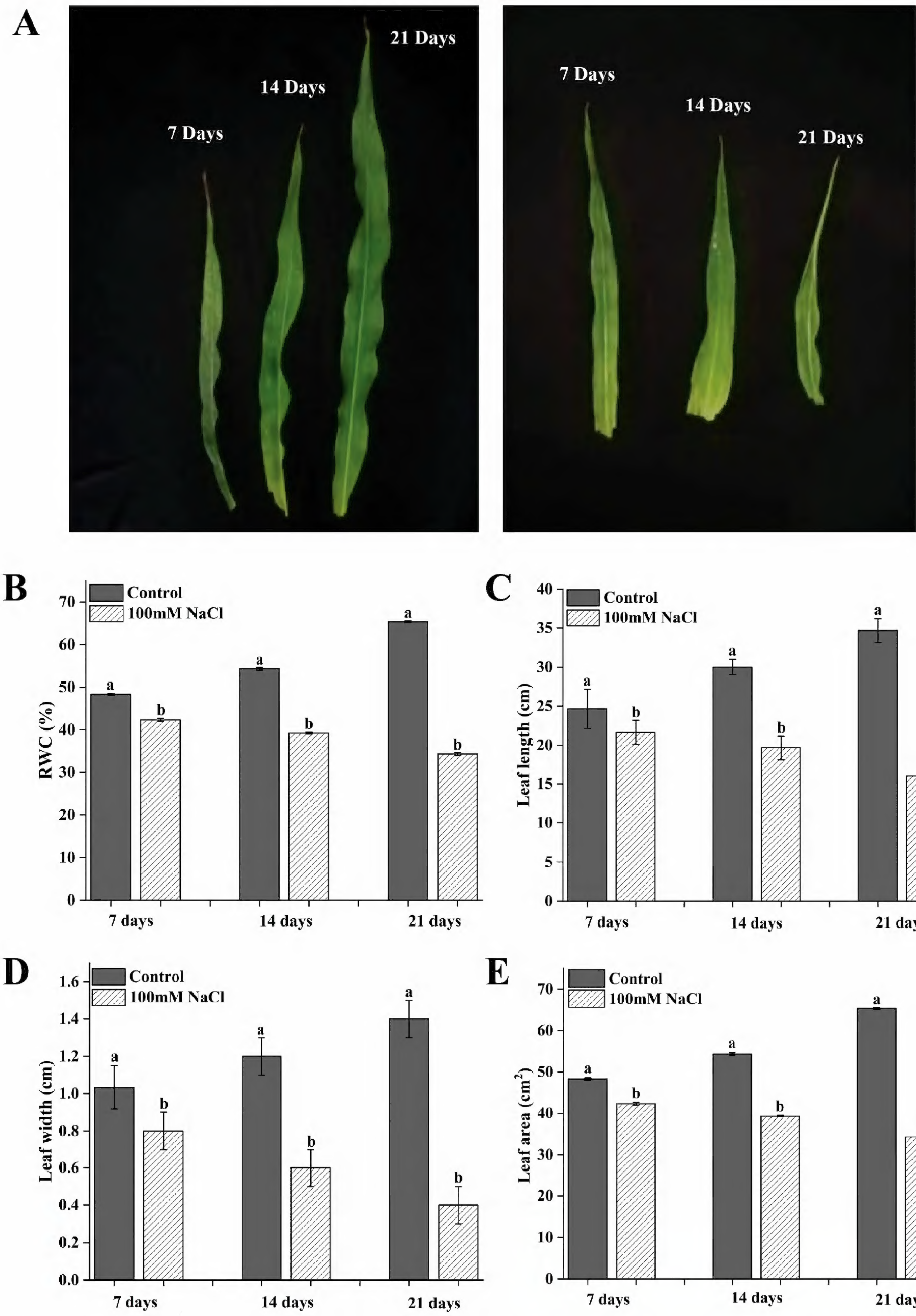

Figure 3. Effect on maize leaves (**A**) RWC (**B**) leaf length (**C**) and (**D**) Leaf width and (**E**) of the maize plant for the control and treatment plants with different periods.

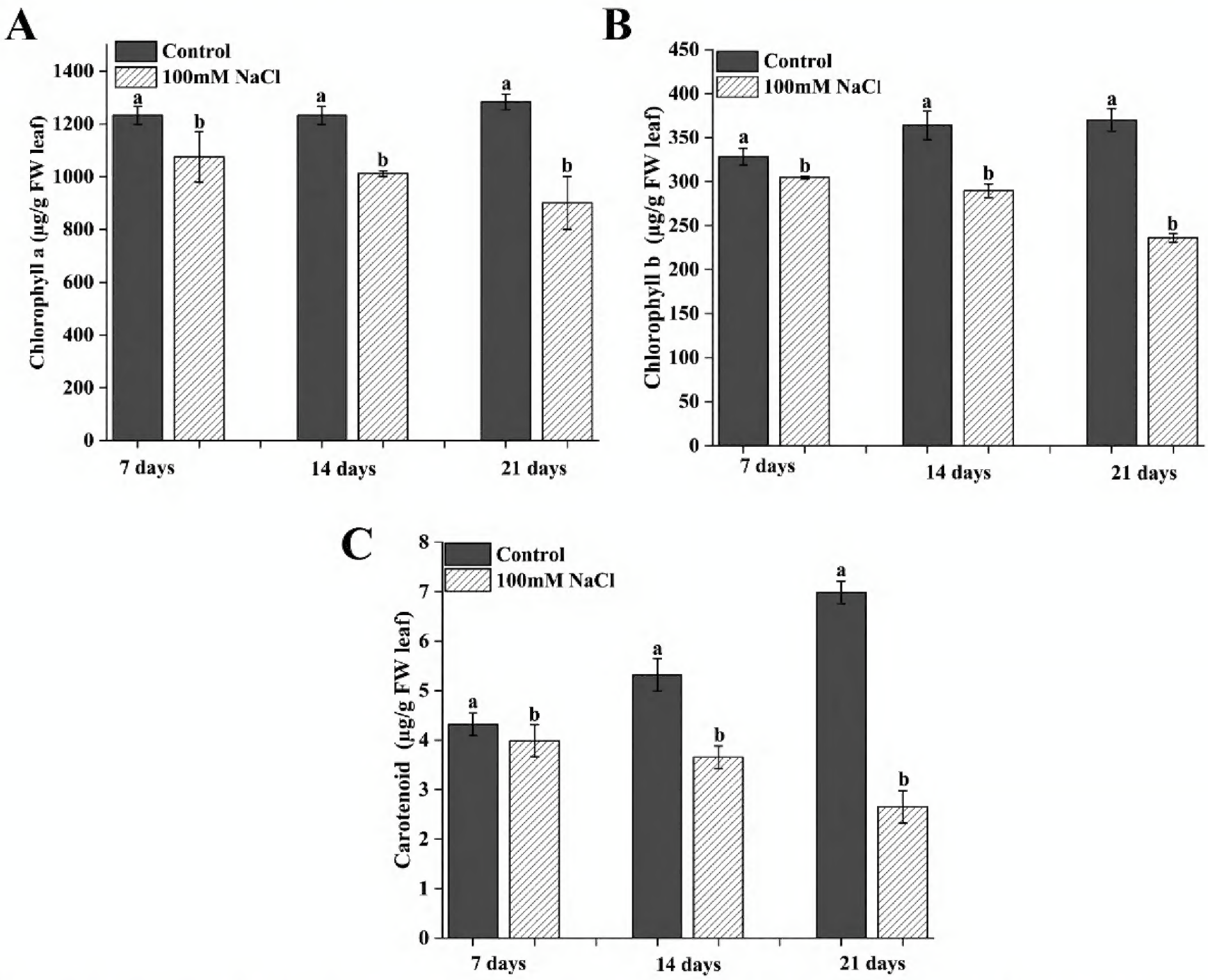

Figure 4. Effect on Chlorophyll content with 7, 14, and 21, days under 100 mM NaCl. Chlorophyll a (**A**). Chlorophyll b (**B**). and Carotenoid (**C**). According to the DMRT, plants treated and in control with varied lengths of salt stress showed significant variations ($p < 0.05$) in bars with distinct small letters.

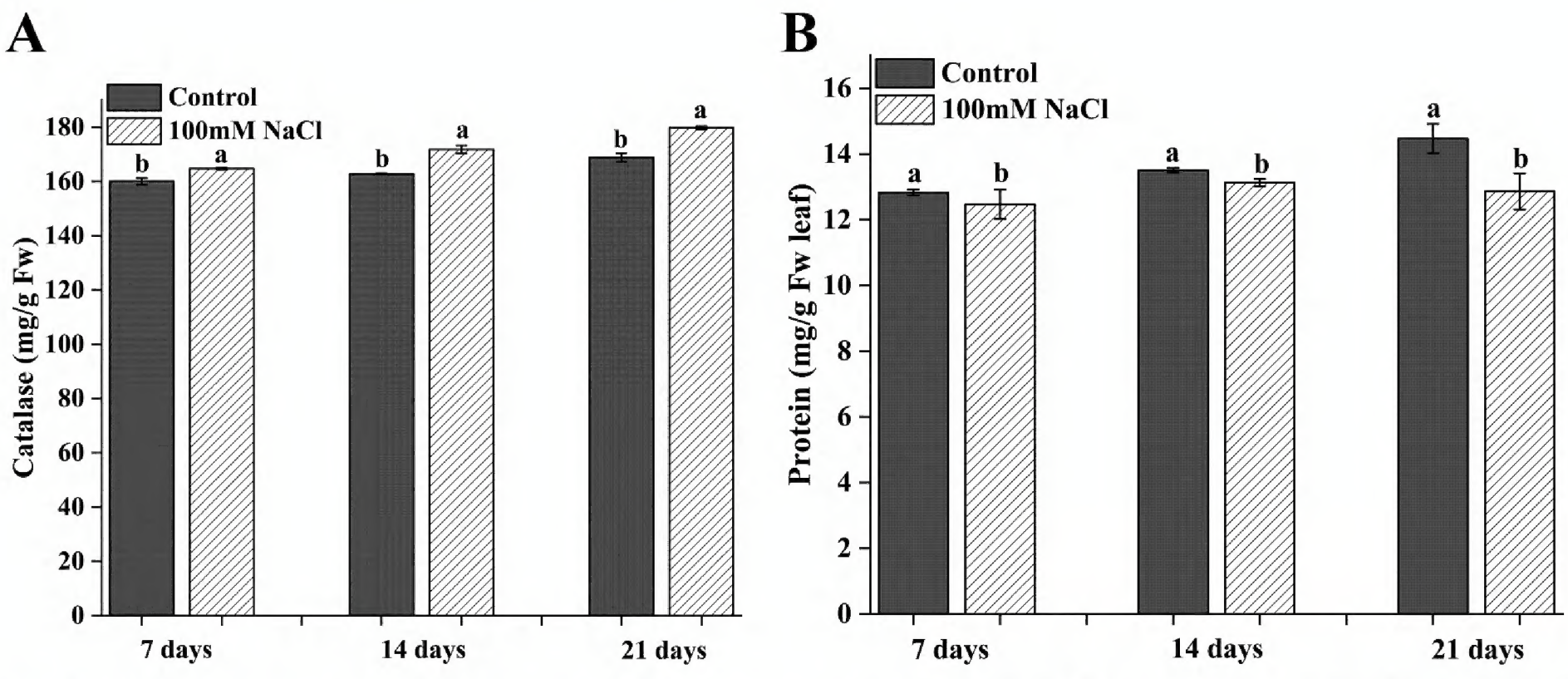

Figure 5. Effect on Catalase and Protein with different periods under 100 mM NaCl. Catalase (**A**) and Protein (**B**). Based on the DMRT, bars containing additional tiny letters denote significant differences ($p < 0.05$) between treated and control plants that experienced different lengths of salt stress.

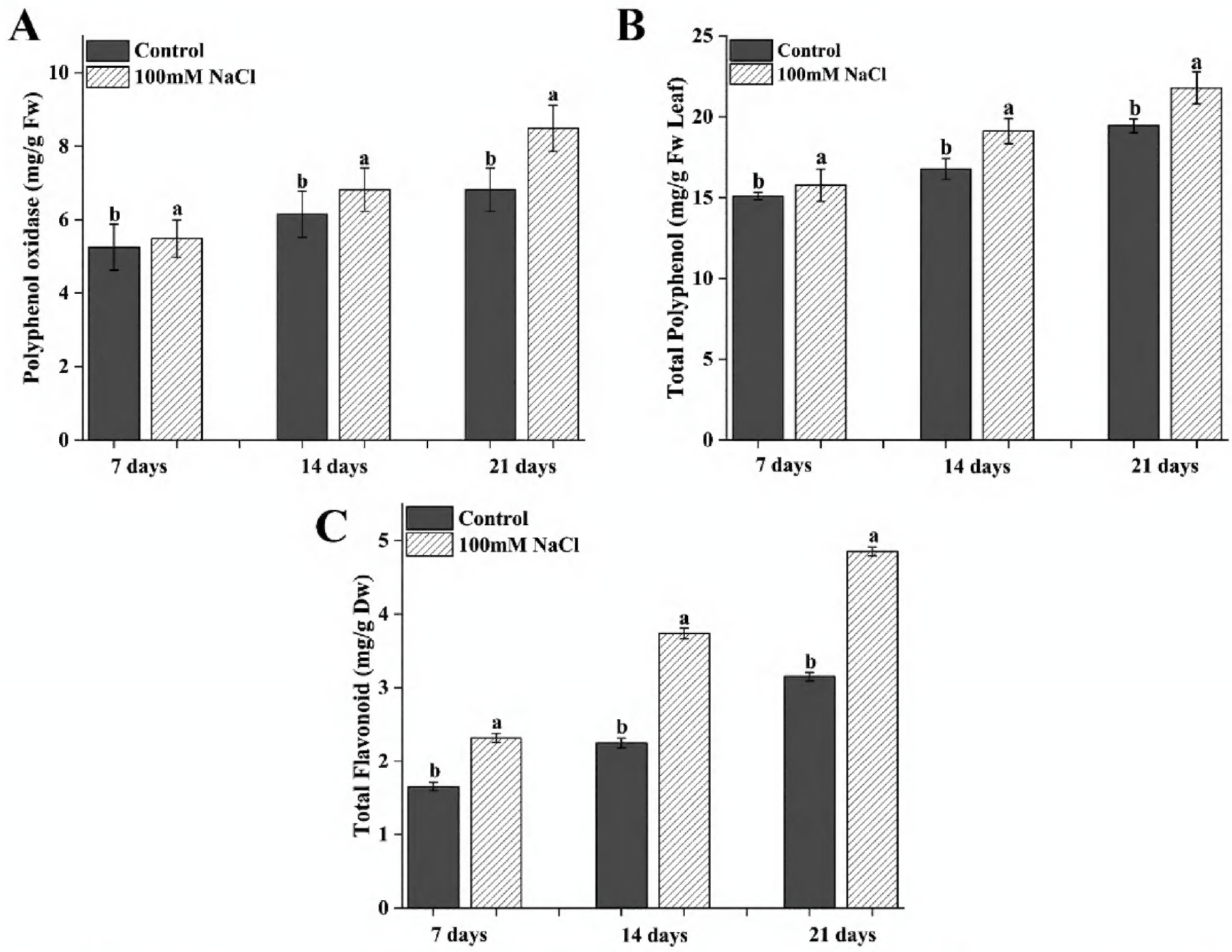

Figure 6. Effect on non-enzymatic contents with different periods under 100 mM NaCl. **A.** Polyphenol oxidase; **B.** Total polyphenol oxidase; **C.** Total Flavonoids. Based on the DMRT, bars containing additional tiny letters denote significant differences ($p < 0.05$) between treated and control plants that experienced different lengths of salt stress.

stress conditions As a result of these findings, PPO, total polyphenol, and flavonoid contents changed over various periods in response to salt stress, demonstrating how plants adapt to stress and highlighting how stress duration relates to these biochemical parameters in nuanced ways.

Principal component analysis

PCA analysis dispersed results data in different periods of plants in three-quarters of biplots (Fig. 7). PCA aims to investigate the optimal number of components that can be extracted to decrease the effective dimensionality. Study collected data from various variables from 7, 14, and 21 days of control and 100 mM NaCl plants. The principal component analysis assessed the impact of salinity stress on plant and leaf biomass, and enzymatic and non-enzymatic activities (Fig. 7). In PCA plots, PC1 consistently had a higher value than PC2. Furthermore, the eigenvalue increased in the control group, it decreased under stress conditions. Similarly, cumulative effects were observed over different periods under salinity stress. At 7, 14, and 21 days with 100 mM NaCl stress conditions, variance values of 0.02%, 0.05%, and 0.18% were observed in biplots (Table 1). The experimental data were based on factor scores and analyses for fourteen plant data in the maize plant exposed to 100 mM NaCl over different periods. The PCA analysis included three variables for both control and stress conditions at 7, 14, and 21 days. We observed catalase contents most resistant found in 100 mM NaCl across different periods whereas, chlorophyll content remained relatively similar at 7 days. Furthermore, the chlorophyll contents a and b, exhibited extreme and opposite changes at 14 and 21 days under the control.

Table 1. Eigen value, Percentage of Variance, and cumulative axes of PCA.

Periods	Treatments	Eigen value	Percentage of Variance	Cumulative
7 days	Control	1.99958	99.98%	99.98%
	100 mM NaCl	0.00230	0.02%	100.00%
14 days	Control	1.99892	99.95%	99.95%
	100 mM NaCl	0.00109	0.05%	100.00%
21 days	Control	1.99646	99.82%	99.82%
	100 mM NaCl	0.00354	0.18%	100.00%

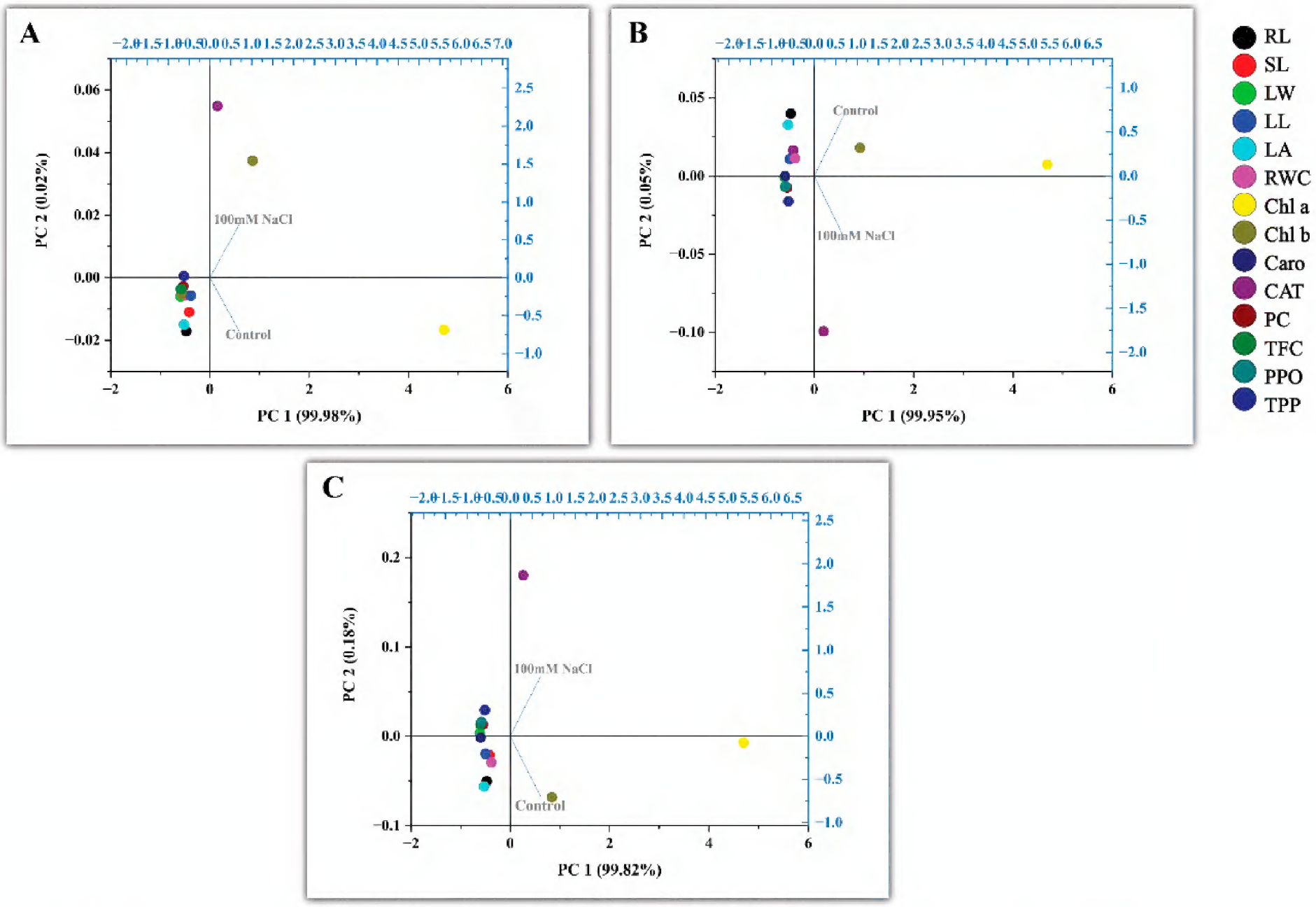

Figure 7. PCA Biplots: **A.** 7 days; **B.** 14 days and **C.** 21 days. RL (root length); SL (shoot length); LW (leaf width); LL (leaf length); LA (leaf area); RWC (relative water content); Chl a (chlorophyll a); Chl b (chlorophyll b); Caro (carotenoid); CAT (catalase); PC (protein contents); TFC (total flavonoid contents); PPO (polyphenol oxidase); TPP (total polyphenol contents).

Discussion

Salt stress is one of the major destructive environmental stress and severely impacting on seed germination, plant biomasses and the overall productivity of plants worldwide (Shah et al. 2021; Song et al. 2023). Subjecting NaCl stress to plants can result in a various range of morphological, physiological, and biochemical changes, which reduced plant development and growth (Nawaz et al. 2010; Ahmad et al. 2022). Previous research has demonstrated that salt stress decreases the development and growth of various plants including maize (El-Naim et al. 2012; Aizaz et al. 2023a), wheat (Aizaz et al. 2023b), rice (Hoang et al. 2016) and sorghum (Nxele et al. 2017). The present investigation is consistent with previous studies that NaCl stress negatively affects maize plants physiologically and biochemically. Our results revealed that like other cereal crops like rice, wheat, and barley NaCl stress significantly impact maize seed germination. In the current study the NaCl stress decreased the plant biomass and chlorophyll contents with time (Fig. 1). Both the shoot and root are very sensitive to NaCl stress, resulting in reduced fresh and dry weight of maize plants agreement with previous results (Saddiq et al. 2019).Changes in plant growth indexes and biomass provide valuable insights into the plants ability to tolerate and adapt to salt stress (Mao et al. 2016; Hadi et al. 2021). Our finding are consistent with previous studies that consistent patterns of decreasing growth indexes and biomass occur with increasing periods of salt treatment (Albacete et al. 2008). Previous studies found similar results, and showed that leaf length and width were reduced due to salinity stress (Kumar et al. 2021). Similarly, current data demonstrates a significant decrease in leaf length and width when exposed to salt stress over different periods compared to control. Additionally, it mentions that salinity stress impacts the relative water content of leaves (Lee et al. 2005; Aizaz et al. 2023a). Our study compared various periods (7, 14, and 21 days) under 100 mM salt stress. We analyzed a significant reduction in the relative water content of leaves and chlorophyll in maize plants compared to the control group across different periods. This reduction in RWC is attributed to salt stress, which leads to lower water availability (Shalhevet 1993). Excessive sodium ion accumulation under salt stress triggers osmotic stress, which alters water potential, interacts with water permeability and decreases cell turgidity (Cramer et al. 2007). Consistently, in the current investigation, we noted an RWC significantly reduced after 21 days of treating plants with 100 mM NaCl, compared to both control and plant treated with NaCl for 7 and 14 days.

The primary indication of the water stress that inhibits the water flow to the new cell elongation sites is a reduction in the RWC of the cells (Haider et al. 2019). Similar effects have been observed in peach (Haider et al. 2019) and tomato (Maggio et al. 2007). It was notable that chlorophyll contents decreased in plants under stress conditions. It could damage the chloroplast structure, which may cause the reduction of chlorophyll contents (Cao et al. 2015) which has previously been reported in rice (Sultana et al. 1999) and maize (Henry et al. 2015). Our findings align with previous studies that reduction in chlorophyll contents and damage to chloroplasts under high salt concentrations result in a significant decrease in the plant's ability to assimilate CO_2, which is a critical component of the photosynthetic process (Dichala et al. 2021; Zhu et al. 2021). Catalase activity observed increased under the stress condition is probably due to an improved capacity to combat oxygen radicals and preserve the integrity of cellular membranes. This implies a connection between salt tolerance and the efficiency of the antioxidant defense mechanism (Mane et al. 2010). The current results showed that with an increase in different periods with same salt concentration, the Catalase activity showed an overall trend to increase, significantly compared to a control with salt concentration. Our results were supported by earlier studies conducted in malus (Wang et al. 2022) and mung bean (Aizaz et al. 2023a). Protein contents decrease with time in salt-treated plants (Doganlar et al. 2010). Salt stress can hurt plant physiology by causing potassium ion removal from plant roots. This disruption in potassium homeostasis can affect various cellular processes, including protein synthesis, ultimately decreasing protein content in salt-treated plants (Kumari et al. 2021). However, the response of plants to salt stress can vary based on their species and genetic makeup. Some salt-sensitive plants may increase their protein content as a stress adaptation strategy to mitigate protein loss and maintain essential cellular functions when exposed to high salt concentrations (Flowers and Yeo 1995). In the current study, a fluctuation in protein content in maize seedlings over different intervals of NaCl treatment was observed. When exposed to NaCl treatment, maize seedlings may initially (1–7 days) respond to the stress by increasing protein synthesis. The increased protein content might help the plant adapt to the initial shock caused by the elevated salt levels (Doganlar et al. 2010). In 2nd week salt stress may interfere with various cellular processes, including protein synthesis as a result, protein content decreases during this period possibly due to the plant's struggles to maintain protein homeostasis in the face of ongoing stress (Ahmad and Sharma 2008; Ullah et al. 2019a; Ullah et al. 2020). In the last seven days interval, an increase in protein content was again observed, which could indicate the plant's attempt to acclimate or recover from the stress. Previous studies also found similar results (Ayala-Astorga and Alcaraz-Meléndez 2010). Plants have developed protective mechanisms to mitigate the damage caused by ROS and maintain cellular homeostasis. These mechanisms include antioxidant enzymes (e.g., superoxide dismutase, catalase, peroxidase) and non-enzymatic antioxidants (e.g., ascorbate, glutathione) that neutralize and scavenge excess ROS (Mishra et al. 2023). Under the salt stress condition, the balance between ROS production and the plant's protective mechanisms may be disrupted, resulting in an accumulation of ROS and subsequent oxidative damage to cellular components (Hasanuzzaman et al. 2020). This oxidative damage can harm the plant's growth, development, and overall health (Huang et al. 2019). Maize exhibited enhanced antioxidant enzyme activities in response to a saline environment, which helped it combat oxidative damage caused by reactive oxygen species (ROS) (Mahmood et al. 2021). Antioxidant enzymes assure cell stability against ROS production under stress conditions. Salt stress tolerance is endorsed to induce antioxidant enzyme activity, thus decreasing oxidative detriment in plant cells (Reddy et al. 2004).

Conclusion

The present study aimed to investigate the detrimental impact of salt stress on maize plants over different growth durations. We have found that NaCl stress significantly decreased the maize shoot and root length and width of leaves. Furthermore, the decline in chlorophyll contents was observed which indicates impairments in the plants' ability to perform photosynthesis, which is crucial for their overall growth. The increased activity of enzymes like catalase and PPO, as well as the higher levels of total phenolic compounds and total flavanols, suggest that the plants were mounting a stress response to combat the effects of salt stress. The current study highlights the critical importance of developing innovative strategies to reduce the adverse effects of salt stress on plant growth and survival. Ensuring the food security of local maize varieties in saline regions is essential. The study will provide valuable insights for addressing these challenges.

Author Contributions

M.A., T.U., performed experimental works, M.A., R.U, M.M.A., A.A., wrote the manuscript, R.U., S.H.Q.M., T.U., M.A., performed the statistical analysis and R.U., R.S., M.M.A., A.A., reviewed and revised the manuscript draft.

Funding

This research was funded by Taif University, Saudi Arabia, Project No. (TU-DSPP-2024-10).

Acknowledgements

The authors extend their appreciation to Taif University, Saudi Arabia, for supporting this work through project number (TU-DSPP-2024-10).

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